=> d 1-4 bib ab

L14 ANSWER 1 OF 4 MEDLINE
AN 2001700894 MEDLINE
DN 21617075 PubMed ID: 11741351
TI Adapting pharmacokinetic properties of a humanized anti-interleukin-8 antibody for therapeutic applications using site-specific **pegylation**.
AU Leong S R; DeForge L; Presta L; Gonzalez T; Fan A; Reichert M; Chuntharapai A; Kim K J; Tumas D B; Lee W P; Gribling P; Snedecor B; Chen H; Hsei V; Schoenhoff M; Hale V; Deveney J; Koumenis I; Shahrokh Z; McKay P; Galan W; Wagner B; Narindray D; Hebert C; Zapata G
CS Department of Immunology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA.. steven.leong@maxygen.com
SO CYTOKINE, (2001 Nov 7) 16 (3) 106-19.
Journal code: 9005353. ISSN: 1043-4666.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200203
ED Entered STN: 20011220
Last Updated on STN: 20020312
Entered Medline: 20020311
AB A neutralizing anti-interleukin-(IL-)8 monoclonal antibody was humanized by grafting the complementary determining regions onto the human IgG framework. Subsequent alanine scanning mutagenesis and phage display enabled the production of an affinity matured antibody with a >100-fold improvement in IL-8 binding. Antibody fragments can be efficiently produced in Escherichia coli but have the limitation of rapid clearance rates in vivo. The Fab' fragment of the antibody was therefore modified with polyethylene glycol (**PEG**) in order to obtain a more desirable pharmacokinetic profile. **PEG** (5-40 kDa) was site-specifically conjugated to the Fab' via the single **free cysteine** residue in the hinge region. In vitro binding and bioassays showed little or no loss of activity. The pharmacokinetic profiles of the 20 kDa, 30 kDa, 40 kDa, and 40 kDa branched **PEG** -Fab' molecules were evaluated in rabbits. Relative to the native Fab', the clearance rates of the **PEGylated** molecules were decreased by 44-175-fold. In a rabbit ear model of ischemia/reperfusion injury, all **PEGylated** Fab' molecules were as efficacious in reducing oedema as the original monoclonal antibody. These studies demonstrate that it is possible to customize the pharmacokinetic properties of a Fab' while retaining its antigen binding activity.

L14 ANSWER 2 OF 4 CAPLUS
AN 2001:851194 CAPLUS
DN 136:2090
TI Methods for refolding of growth hormone supergene family **proteins** containing **free cysteine** residues
IN Rosendahl, Mary S.; Cox, George N.; Doherty, Daniel H.
PA Bolder Biotechnology, Inc., USA
SO PCT Int. Appl., 110 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001087925	A2	20011122	WO 2001-US16088	20010516
	WO 2001087925	A3	20020801		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1284987 A2 20030226 EP 2001-941504 20010516
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

PRAI US 2000-204617P P 20000516
WO 2001-US16088 W 20010516

AB The present invention relates to novel methods for making and refolding insol. or aggregated **proteins** having free cysteines in which a host cell expressing the **protein** is exposed to a cysteine blocking agent. Blocking of free cysteines prevents the crosslinking of **proteins** into large insol. aggregates, keeping them in soln. and simplifying purifn. and increasing the yield of the biol. activity. The sol., refolded **proteins** produced by the novel methods can then be modified to increase their effectiveness. Such modifications include attaching a **PEG** moiety to form **PEGylated proteins**. The **PEGylated proteins** of the investigation include recombinant cysteine variants of members of the growth hormone supergene family such as: growth hormone, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, and .alpha.-interferon.

L14 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS
AN 2000:493669 CAPLUS
DN 133:116453
TI Methods for making **proteins** containing **free cysteine** residues using thiol protective agents in culture media
IN Cox, George N.; Doherty, Daniel H.; Rosendahl, Mary S.
PA Bolder Biotechnology Inc., USA
SO PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000042175	A1	20000720	WO 2000-US931	20000114

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

CA 2359345	AA	20000720	CA 2000-2359345	20000114
EP 1144613	A1	20011017	EP 2000-902412	20000114

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

BR 2000008759	A	20020806	BR 2000-8759	20000114
JP 2002534119	T2	20021015	JP 2000-593732	20000114

PRAI US 1999-116041P P 19990114
WO 2000-US931 W 20000114

AB The present invention relates to novel methods of making sol.

proteins having free cysteines in which a host cell is exposed to a cysteine blocking agent. Blocking of free cysteines prevents the crosslinking of **proteins** into large insol. aggregates, keeping them in soln. and simplifying purifn. and increasing the yield of the biol. activity. The sol. **proteins** produced by the methods can then be modified to increase their effectiveness. Such modifications include attaching a **PEG** moiety to form **PEGylated proteins**. The method can be used with intracellular or secretory expression systems and involves adding a thiol protective agent to the culture medium, either during the culture process or immediately before cell lysis. The use of cystine to prevent aggregation of analogs of human growth hormone contg. addnl. cysteines is demonstrated.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS
AN 1997:164112 CAPLUS
TI Issues encountered in the production of site-specific mono-**PEGylated** therapeutic **proteins**.
AU Seely, J.; Richey, C.; Grasel, T.; Wilson, J.
CS Amgen Process Development, Boulder, CO, 80301, USA
SO Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17 (1997), POLY-187 Publisher: American Chemical Society, Washington, D. C. CODEN: 64AOAA
DT Conference; Meeting Abstract
LA English
AB Adding a single **PEG** mol. to a **protein** can markedly extend its serum half-life. By directing where the mono-**PEGylation** occurs, we can reduce the clearance rate without adversely affecting the biol. activity of the **protein**. We have used **PEG** vinylsulfone for site-specific modification at **free cysteine** residues and **PEG** aldehyde for selective modification of the N-terminal alpha amino group. Several issues have been encountered that effect both the yield and quality of monoPEGylated **proteins**. These issues include **PEG** linker purity (both in terms of the degree of activation and size homogeneity), **PEGylation** conditions, **PEG** linker stability and, in the case of **PEG** aldehyde, sodium cyanoborohydride quality. Examples of each of these will be presented, as will some of the ways in which these problems have been addressed.

ANSWER 2 OF 2 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.

AN 2003051375 EMBASE

TI Effective drug delivery by **PEGylated** drug conjugates.

AU Greenwald R.B.; Choe Y.H.; McGuire J.; Conover C.D.

CS R.B. Greenwald, Enzon, Inc., 20 Kingsbridge Road, Piscataway, NJ 08854-3969, United States. richard.greenwald@enzon.com

SO Advanced Drug Delivery Reviews, (10 Feb 2003) 55/2 (217-250).
Refs: 133
ISSN: 0169-409X CODEN: ADDREP

PUI S 0169-409X(02)00180-1

CY Netherlands

DT Journal; General Review

FS 037 Drug Literature Index
039 Pharmacy

LA English

SL English

AB The current review presents an update of drug delivery using poly(ethylene glycol) (**PEG**), that focuses on recent developments in both **protein** and organic drugs. Certainly the past 10 years has resulted in a renaissance of the field of **PEG** drug conjugates, initiated by the use of higher molecular weight **PEGs** (M(w)>20,000), especially 40,000 which is estimated to have a plasma circulating t(1/2) of approximately 10 h in mice. This recent resuscitation of small organic molecule delivery by high molecular weight **PEG** conjugates was founded on meaningful in vivo testing using established tumor models, and has led to a clinical candidate, **PEG** -camptothecin (PROTHECAN.RTM.), an ester based prodrug currently in phase II trials. Additional applications of high molecular weight **PEG** prodrug strategies to amino containing drugs are presented: similar tripartate systems based on lower M(w) **PEG** and their use with **proteins** is expounded on. The modification of a benzyl elimination tripartate prodrug specific for mercaptans is presented, and its successful application to 6-mercaptopurine giving a water **soluble** formulation is discussed. Recent novel **PEG** oligonucleotides and immunoconjugates are also covered. Clinical results of FDA approved **PEGylated proteins** are also presented.